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Synthesis Of Silicon Precursors Of Modified Oligonucleotides

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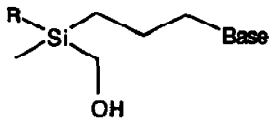
Abstract: The synthesis of four silicon nucleoside analogues for use as modified antisense oligonucleotide precursors, is described.

In the field of antiviral or anticancer chemotherapy, the use of antisense oligonucleotides is increasingly promising [1,2]. Expression of viral proteins can be inhibited by the base-pairing of oligonucleotide sequences complementary to the coding sense strand of viral mRNA [3,4]. Such strands can be made of a small number of nucleotides (< 20) with a high specificity [5], and can be synthesized with automated DNA synthesizers. Nevertheless, there are limiting factors for the therapeutic use of natural antisense oligonucleotides such as degradation by nucleases, and low cellular uptake due to their strong anionic nature. To overcome these difficulties, the synthesis of oligonucleotides chemically modified in either the phosphodiester or ribofuranose components has been undertaken (see ref. 1 for a review). Recently, modified oligonucleotides have been synthesized in which the ribofuranose moiety has been replaced by 2-aminoethyl glycine units [6].

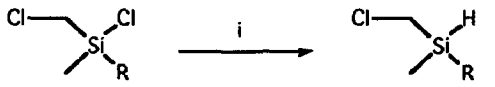
Our strategy, is to incorporate within an antisense oligonucleotide, nucleoside analogues consisting of organosilicon monomeric units. We expect from the presence of silicon, to increase the overall lipophily and to exploit modification of the fourth valency on the silicon atom for various purposes (improved solubility, cellular diffusion, ...). We report here the synthesis of four such organosilicon compounds (**1a-d**) bearing thymine and adenine nucleobases and one or two hydroxymethyl groups attached to the silicon atom *via* the phosphoramidite protocol for the solid phase polymer synthesis.



1a:	R= CH_2OH	Base= Thymine
1b:	R= Me	Base= Thymine
1c:	R= CH_2OH	Base= Adenine
1d:	R= Me	Base= Adenine

The key step of this synthesis lies in a hydrosilylation reaction between an N-allyl nucleobase and a

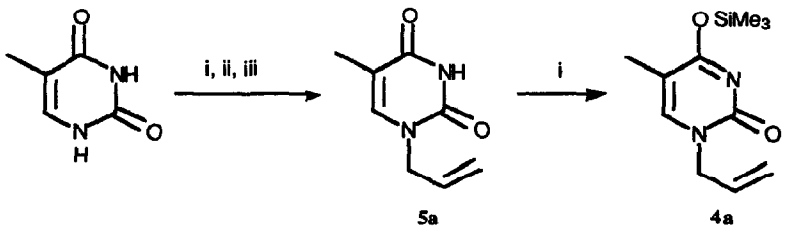
suitable silane prepared by reduction of the corresponding chlorosilane [7] (Scheme 1).



R= CH_2Cl (93%)
R= CH_3 (78%)

Scheme 1 - i: $LiAlH_4$, Et_2O, 0°C

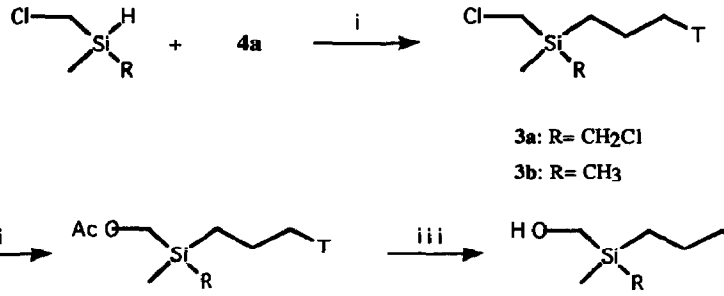
For the preparation of the target molecules **1a** and **1b**, thymine was derivatized as its bis(trimethylsilyloxy) derivative, alkylated with allyl bromide to give a mixture of N-1-allylthymine [8] **5a** (65%) and N,N-1,3-bis(allyl)thymine (15%) as the only detected by-product. Finally, we found more convenient to protect **5a** as its trimethylsilyl ether **4a** since the subsequent hydrosilylation reaction was more efficient in the absence of the labile N-3 proton (Scheme 2).



5a **4a**

Scheme 2 - i: HMDS, $(NH_4)_2SO_4$; ii: allyl bromide/DMF, 80°C, 72h; iii: H_2O, 0°C.

Thus, **4a** was reacted with bis(chloromethyl)methylsilane or bis(methyl)chloromethylsilane with chloroplatinic acid as catalyst to yield **3a** (35%) and **3b** (20%) respectively (Scheme 3).



3a: R= CH_2Cl
3b: R= CH_3

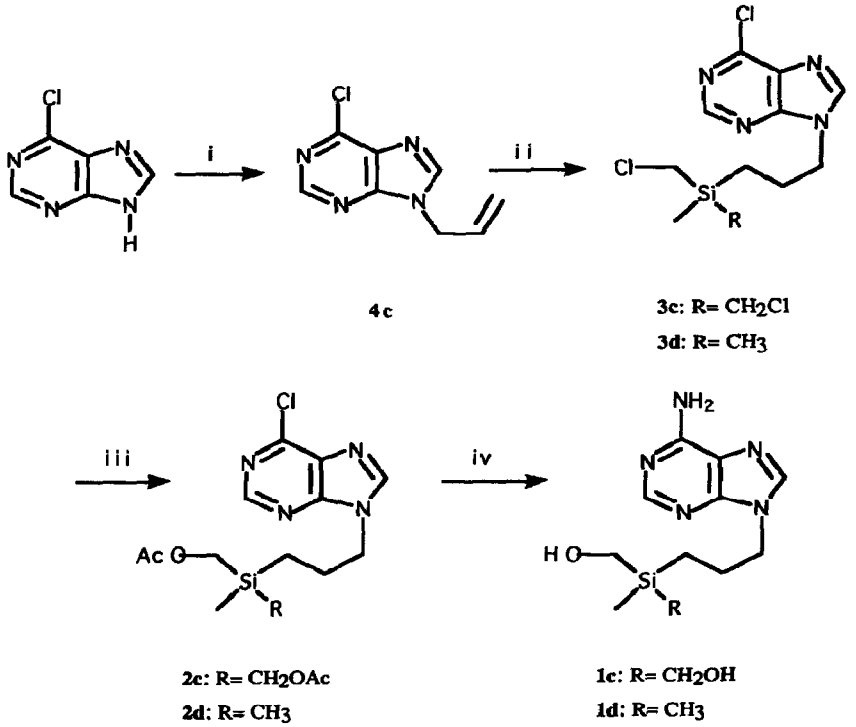
2a: R= CH_2OAc
2b: R= CH_3

1a: R= CH_2OH
1b: R= CH_3

Scheme 3 - i: 1) H_2PtCl_6, THF 2) H_2O; ii: AcONa, DMF, iii: 1) K_2CO_3, MeOH 2) HCl 1N.

The other steps of the synthesis involved the nucleophilic displacement of the chloromethyl compounds **3a** and **3b** with sodium acetate to give the ester precursors **2a** (72%) and **2b** (54%). Subsequent saponification (MeOH, K_2CO_3) afforded **1a** (76%)[9] and **1b** (60%)[10].

Compounds **1c** and **1d** (base= adenine) were prepared as outlined below (Scheme 4).



Scheme 4 - i: 1) NaH/DMF 2) allyl bromide, DMF; ii: 1) $ClCH_2(Me)SiH$ or $(ClCH_2)_2(Me)SiH$, H_2PtCl_6, THF 2) H_2O; iii: AcONa/DMF; iv: NH_3/MeOH.

We started from the readily available 6-chloropurine [11], as its allyl derivative **4c** gave better yields of hydrosilylation products than allyladenine we used in our pilot studies (average yield 60% *vs.* 3%). The sodium salt of 6-chloropurine was alkylated with allyl bromide to afford **4c** (65%) which, upon hydrosilylation, yielded **3c** (57%) and **3d** (17%). Compounds **3c** and **3d** were acetoxylated to give **2c** (66%) and **2d** (not isolated) which were further reacted with methanolic ammonia to yield the adenyl derivatives **1c** (65%)[12] and **1d** (50% from **3d**)[13].

The synthesis of poly-T and poly-A oligodeoxynucleotides including one or several **1a-d** monomeric units is currently underway, and our results will be reported later.

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9. Satisfactory spectroscopic data (FT-IR, 200 MHz ^{1}H and 50 MHz ^{13}C NMR) were obtained for all compounds. **1a**, mp= 116 °C, ^{1}H NMR (CD_3OD), δ (ppm) 0.07 (s, 3H, SiMe), 0.62-0.71 (m, 2H, $SiCH_2$), 1.66-1.82 (m, 2H, Si-C-CH_2), 1.85 (d, 3H, $^4J_{Me,H-6}$= 1 Hz, Me of thymine), 3.43 (s, 4H, 2 C$\underline{H}_2$OH), 3.69 (t, 2H, J= 7.3 Hz, CH_2N), 7.42 (d, 1H, H-6); ^{13}C NMR (CD_3OD), δ (ppm) -8.25 (SiMe), 8.34 ($SiCH_2$), 12.20 (Me of thymine), 24.17 (Si-C-$\underline{C}$), 52.13 (CH_2N), 52.50 (CH_2OH), 110.86 (C-5), 143.22 (C-6), 152.82 (C-2), 166.76 (C-4).
10. Compound **1b**, mp= 138 °C, ^{1}H NMR ($CDCl_3$), δ (ppm) -0.01 (s, 6H, $SiMe_2$), 0.49-0.58 (m, 2H, $SiCH_2$), 1.58-1.74 (m, 2H, Si-C-CH_2), 1.85 (d, 3H, $^4J_{Me,H-6}$= 1 Hz, Me of thymine), 2.16 (large s, exchanged with D_2O, 1H, OH), 3.35 (s, 2H, C$\underline{H}_2$OH), 3.62 (t, 2H, CH_2N), 6.99 (d, 1H, H-6), 9.94 (large s, 1H, NH); ^{13}C NMR ($CDCl_3$), δ (ppm) -5.09 ($SiMe_2$), 10.42 (Si-C), 12.39 (Me of thymine), 23.61 (Si-C-$\underline{C}$), 51.48 (CH_2N), 54.96 (CH_2OH), 110.57 (C-5), 140.62 (C-6), 150.96 (C-2), 164.26 (C-4).
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12. Compound **1c**, mp= 146 °C, ^{1}H NMR (DMSO-d_6), δ (ppm) -0.06 (s, 3H, SiMe), 0.46-0.54 (m, 2H, $SiCH_2$), 1.79-1.87 (m, 2H, Si-C-CH_2), 3.17 (s, 4H, 2 C$\underline{H}_2$OH), 3.96 (large s, exchanged with D_2O, 2H, 2 OH), 4.08 (t, 2H, CH_2N), 7.29 (s, exchanged with D_2O, 2H, NH_2), 8.13 (2s, 2H, H-2 and H-8); ^{13}C NMR (DMSO-d_6), δ (ppm) -7.93 (SiMe), 7.74 (Si-C), 24.11 (Si-C-$\underline{C}$), 45.91 (CH_2N), 50.22 (CH_2OH), 118.74 (C-5), 141.11 (C-8), 149.54 (C-4), 151.84 (C-2), 155.51 (C-6).
13. Compound **1d**, mp= 166-170 °C, ^{1}H NMR ($CDCl_3$), δ (ppm) 0.04 (s, 6H, $SiMe_2$), 0.55-0.63 (m, 2H, $SiCH_2$), 1.86-2.01 (m, 3H, Si-C-CH_2 + OH), 3.40 (s, 2H, C$\underline{H}_2$OH), 4.19 (t, 2H, J= 7 Hz, CH_2N), 5.76 (large s, exchanged with D_2O, 2H, NH_2), 7.79 (s, 1H, H-8), 8.34 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$), δ (ppm) -5.17 (SiMe), 10.56 (Si-C), 24.74 (Si-C-$\underline{C}$), 46.69 (CH_2N), 54.40 (CH_2OH), 140.41 (C-8), 152.84 (C-2), 155.33 (C-6).

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